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CONTENTS

PHYSIOLOGIC SPECIALIZATION AND VARIATION IN *HELMINTHOSPORIUM GRAMINEUM* RAB.[1]

J. J. CHRISTENSEN and T. W. GRAHAM[2]

INTRODUCTION

Helminthosporium gramineum Rab. causes a destructive disease on barley known as barley stripe. The disease is particularly important in northern barley-growing regions. It is present every year in Minnesota, and in some years causes extensive damage. It is not uncommon to find fields with 5 to 10 per cent of the plants infected, while fields with more than 50 per cent of infected plants have been observed.

Altho barley stripe can be controlled effectively by proper seed treatment, it obviously would be more desirable and economical to control it by means of resistant varieties. All varieties commonly grown in Minnesota, except Trebi, are more or less susceptible. Trebi, however, is not desirable for malting. Therefore, breeding of suitable varieties must be resorted to. But, before breeding of resistant varieties can be put on a sound basis, it is important to know the relative number, distribution, and stability of physiologic forms and their parasitic behavior on the varieties commonly grown in the upper Mississippi Valley.

It has been known for some time that many new varieties of barley, when first grown, appear to be resistant to stripe, but after they have been grown extensively they appear to lose their resistance. This was true of Svansota and Velvet, produced in Minnesota, but perhaps the best example is the behavior of Prentice barley in Denmark. This variety remained resistant for many years but eventually became heavily attacked[3] and has now been displaced by new Danish varieties.

This apparent loss of resistance can best be explained by the appearance of new physiologic forms of *H. gramineum*. In 1925, Johnson (17) found that there were at least two races of *H. gramineum* which responded differently to temperature, and he suggested that there might be parasitic races also. In 1930, Isenbeck (16) proved that there were three, or possibly more, parasitic races of this organism. More recently,

[1] The investigations were supported in part by a grant from the Graduate School of the University of Minnesota.

[2] The writers are greatly indebted to Prof. E. C. Stakman for his help in the preparation of the manuscript and to Dr. E. L. LeClerg for aid in statistical analysis.

[3] This information was supplied by Prof. Ö. Winge, Klg. Veterinaer-og Landbohøjskolés, Arvelighedslaboratorium, Copenhagen, Denmark, who obtained it from phytopathological reports for the years 1909-1924, as published in Volumes 15 to 31 of Tidsskrift for Planteavl.

Christensen and Graham (4) obtained evidence that *H. gramineum* comprises a large number of cultural and parasitic races.

H. gramineum, then, is important practically and scientifically. However, certain difficulties are encountered in studying its pathogenicity and stability. *H. gramineum* causes systemic infection unlike other species of *Helminthosporium* (Figure 1) and behaves more nearly like an obligate parasite. Altho it grows readily in culture, it rarely, if ever, fructifies on artificial media. Therefore, it is necessary to make inoculations with mycelial cultures. Plants are susceptible only until about the time of the emergence of the first leaf, and the temperature range favorable for infection is rather limited. These conditions, however, minimize the chances of contamination between races. Because of the systemic nature of the disease and the very definite symptoms it causes, diseased plants can be distinguished clearly from noninfected plants and from those attacked by other pathogenes. Accurate data on percentages of infection are therefore more readily obtained than with other species of *Helminthosporium* that attack barley.

Fig. 1. Symptoms of Barley Stripe on Seedlings and Mature Plants
Note the characteristically shredded leaves and dwarfed head.

The specific objects of the present investigation were: (1) To determine the relative number and distribution of races of *H. gramineum* and to study their stability on artificial media and on the host; (2) to determine varietal resistance of barley to numerous races of *H. gramineum.*

TERMINOLOGY

Stakman (21), in his review of physiologic specialization in fungi, gives a number of synonyms for the term physiologic form, viz., racial strain, biologic form, parasitic strain, physiologic race, etc. In this paper, the term "race" is used for culturally or parasitically distinct biotypes or very closely related biotypes within the species, *Helminthosporium gramineum*. Each race was designated by number as soon as it was proven to differ clearly and consistently from other races in any character. Races have been designated "cultural races" or "parasitic races," depending on whether they are distinguished by cultural characters or by pathogenicity.

Therefore, the cultural races mentioned in these studies should not be regarded as synonymous with parasitic races, since the former may differ only in cultural characters. Parasitic races are not referred to as such until differences in parasitic capabilities have been demonstrated.

Whenever a variant was isolated and studied in considerable detail, it was given the number of its parent followed by a dash and an additional number. Thus 16-2 indicates the second variant isolated from race 16 growing on a nutrient medium. Variants obtained from monosporous reisolations from the host were designated in the same manner except for the addition of the letter "h" preceding the last number. Thus, 14-h3 indicates the third variant derived from race 14 after passing it back through the host.

MATERIALS AND METHODS

More than 100 monosporous isolations of *H. gramineum* were made from collections of diseased barley obtained from 12 different states of the United States and from two provinces of Canada.[4] In addition, cultures from Germany and Denmark were obtained through the courtesy of Dr. Karl Isenbeck, of the University of Halle, Halle-Saale, Germany. Isolations were made from 86 American collections in 1931, from 15 in 1932, and from 5 in 1933. In most cases 4 or more monosporous cultures were isolated from each collection. If isolates from a single collection proved to be culturally alike, all but one were discarded. The isolates, when found to be distinct culturally, were numbered with Arabic numerals.

Single spores were isolated by means of a micromanipulator. Dry spores from the infected leaves were dusted on a clean, dry coverglass, and were then picked singly and transferred to agar drops in a manner

[4] Most of the collections made outside of Minnesota were furnished by H. G. Ukkelberg. Others were furnished by O. S. Aamodt, C. C. Allison, I. L. Forbes, F. J. Greaney, Lee Hines, L. W. Melander, H. A. Rodenhiser, E. C. Stakman, G. H. Starr, and W. G. Stover.

similar to that described by Dickinson (6) and Hanna (13). Stock cultures usually were grown on one per cent potato-dextrose agar. This medium also was used in most cultural studies. Comparative tests were made chiefly either in 250 cc. Erlenmeyer flasks containing about 30 cc. of medium or in 9 cm. petri dishes containing about 20 cc. of medium.

Seed of the different varieties of barley used in experiments on pathogenicity were obtained from the Division of Plant Genetics and Agronomy of the University of Minnesota. The seed was treated one to two hours with a one-half of one per cent solution of Semesan or Ceresan and then washed two to four hours in running water and subsequently allowed to dry thoroly before sowing.

In studies of pathogenicity, mycelial cultures of the organism were used to inoculate the seed. The inoculum was usually increased by growing the organism on a half-and-half mixture of sterilized wheat and oat seeds placed in pint jars or Erlenmeyer flasks. In a few cases the inoculum was increased on nutrient agar. After 10 to 15 days the inoculum was scattered over and in direct contact with barley seed sown in wooden flats or in pots. The soil used in the pathogenicity tests was previously steamed three to four hours at 12 pounds pressure. After planting, the containers were held at low temperatures, alternating from 10 to 20 degrees C. or at a constant temperature of 15 degrees C. for 7 to 10 days, after which the seedlings were transplanted to the field or to pots placed on greenhouse benches.

EXPERIMENTAL RESULTS

Cultural Races

Previous investigations by the authors (4) and others (16, 17) have shown that there are distinct cultural races of *H. gramineum,* but as yet no extensive study has been made of the cultural behavior of a large number of races. Stakman (21) has pointed out that cultural differences in parasitic fungi may serve as an aid in recognizing parasitic races. Extensive cultural tests were made to obtain information concerning physiologic specialization, variability, and physiology of the stripe organism. Cultural studies were made of approximately 1200 monosporous isolates. Many duplicates were eliminated after comparison in test tubes, but those that appeared different were tested further in petri dishes or in Erlenmeyer flasks.

Type of growth.—In a preliminary test 29 races were compared on potato-sucrose agar in triplicate 9 cm. petri dishes (Table 1). Each petri dish contained about 20 cc. of nutrient agar and all plates were poured at the same time from test tubes previously sterilized at 17 pounds pressure for 25 minutes. All 29 races were distinctly different

from each other. The most important cultural characters of 13 of the 29 races studied are given in Table 1. Since these 13 races are representative, a detailed description of all 29 seems unnecessary.

The type of growth in some races was appressed and inconspicuous (race 46); in others, felt-like (race 44), cottony (race 60), striated (race 21), zonated (race 80), or counterclockwise growth of the aerial mycelium (race 51). The amount of aerial mycelium varied from scant, as in races 46 and 51, to abundant, as in races 16 and 44. The color of mycelium varied from gray to black and from lilac to dark red. There was even greater variation in the color of the substratum: Some races turned the medium black (race 44); others, purple (race 51); others, red (race 80); and still others did not discolor the substratum at all (races 75 and 86).

Because of these striking cultural differences between the 29 races, a much more extensive study was made. In one test 86 races were grown in quadruplicate petri dishes containing 20 cc. of potato-dextrose agar. It is noteworthy that of the 86 races studied, 49 from Minnesota collections and the rest from different regions in the United States and Canada, all but two were distinctly different on this one medium.

Table 1

Cultural Characteristics of 13 Races of *Helminthosporium gramineum* on Potato-Sucrose Agar*

Race No.	Source	Type of growth	Relative amount of aerial mycelium	Color	
				Mycelium	Substratum
10	Minnesota	Appressed, with radial strands†	++	Black to purple†	Black to olive
16	Minnesota	Fluffy	++++	Gray to black	Faint bluish
21	Minnesota	Velvety; striated	++	Light red to purple	Purple
44	Minnesota	Uniformly felty	++++	Dark gray	Black
46	Minnesota	Appressed; inconspicuous	±	Light red	Light red
51	Iowa	Appressed; felty; counterclockwise	+	Gray to black	Dark purple
55	Nebraska	Patchy	+++	Grayish to dark red	Slightly purple
60	Missouri	Cottony	++++	Gray to black	Slightly black
66	South Dakota	Slightly appressed; cottony; radial strands	++	Lilac purple	Lilac to purple
75	North Dakota	Felty; irregular	+++	Grayish	Colorless
80	Wisconsin	Felty to cottony;† zonated	++	Gray to red	Deep red
85	Canada	Cottony to felty	+++	Gray to black	Grayish
86	Minnesota (Infected seed from Germany)	Fluffy, cottony to felty	++++	Gray to black	Colorless

* Notes taken 24 days after inoculation.

† Where more than one color or type of growth is given, the first one mentioned is predominant.

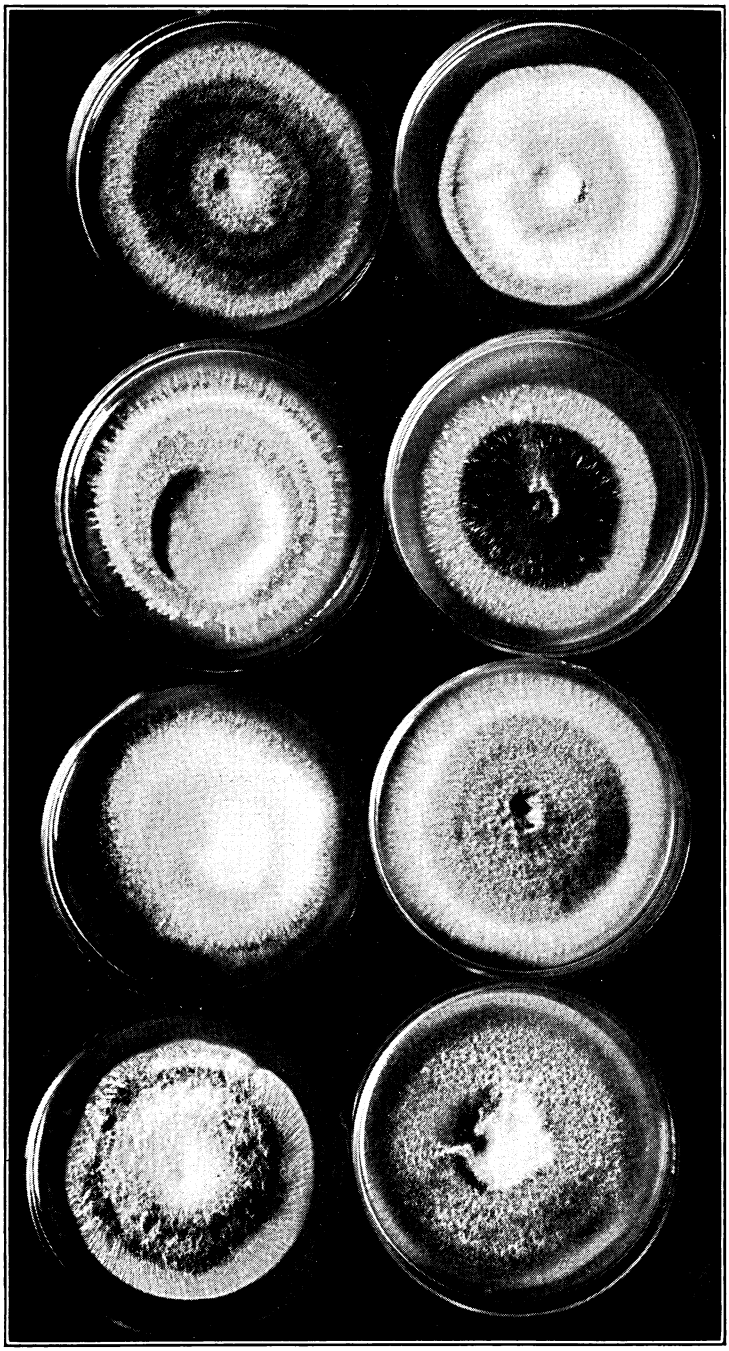

Fig. 2. Eight Races of *H. gramineum* isolated from Minnesota, Nebraska, and North Dakota Collections, Grown for 12 Days on Potato-Dextrose Agar.
Left row, top to bottom,—Races 47 (Minn.), 14 (Minn.), 76 (N.D.), and 36 (Minn.).
Right row, top to bottom,—Races 12 (Minn.), 18 (Minn.), 57 (Nebr.), and 34 (Minn.).

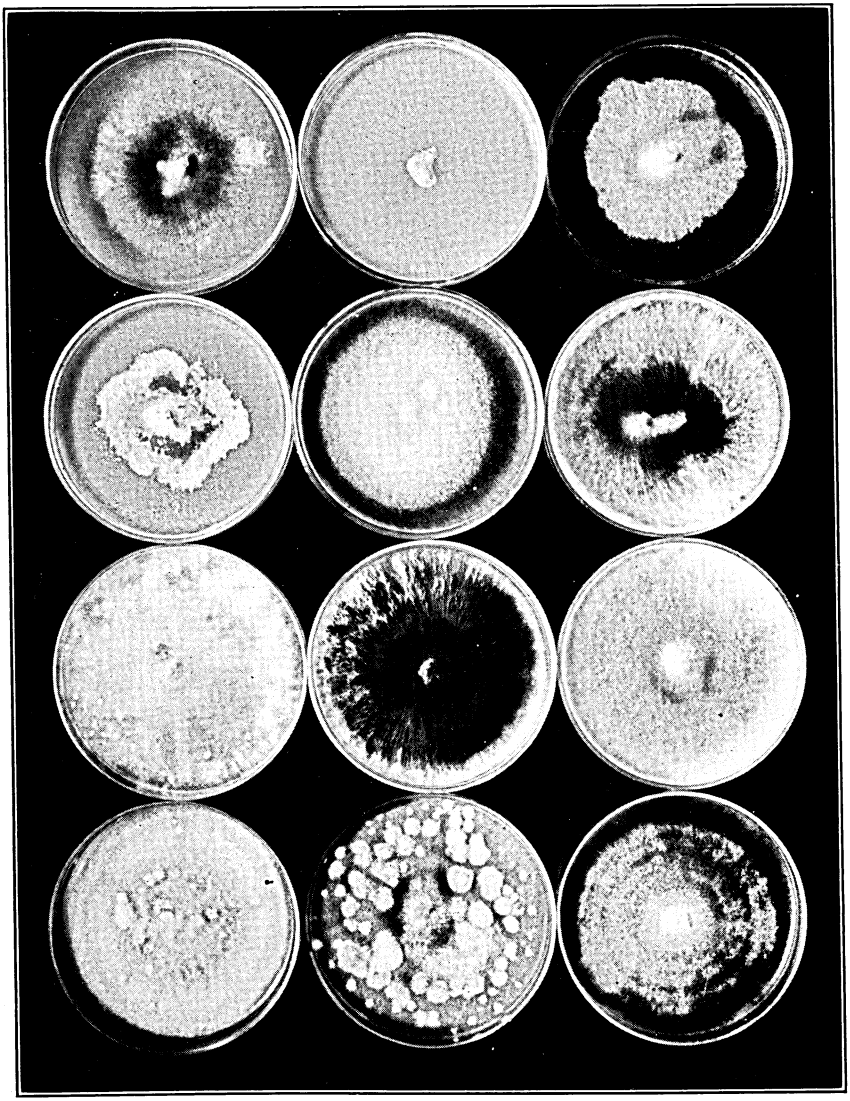

Fig. 3. Twelve Races of *H. Gramineum* Isolated from Minnesota Collections of Barley Stripe, Grown for 12 Days on Potato-Dextrose Agar
Left row, top to bottom,—Races 16, 37, 23, 30.
Middle row, top to bottom,—Races 46, 25, 15, 21.
Right row, top to bottom,—Races 39, 42, 29, 4.

When these two apparently similar races were grown on another medium, they, too, were distinctly different from each other. These 86 races were distinguished from each other principally by the following cultural characters: color of mycelium, discoloration of the medium, type of growth, amount of aerial mycelium, rate of growth, and tendency to sector and to produce "patch" variants.

Table 2 summarizes the characteristics of 20 representative races of the 86 studied under comparable conditions. Within the group from Minnesota, races 26, 46, and 47 have appressed mycelium; yet each differed from the others, in certain other characters. The marked diversity between races can perhaps best be appreciated by looking at Figures 2 and 3. The "paddle wheel" type of growth was characteristic of race 40 (Figure 4), while race 21 could be differentiated by its tendency to produce "patch-like" growths over colonies (Figure 3). In others radial strands or counterclockwise growth of aerial mycelium were outstanding cultural differences.

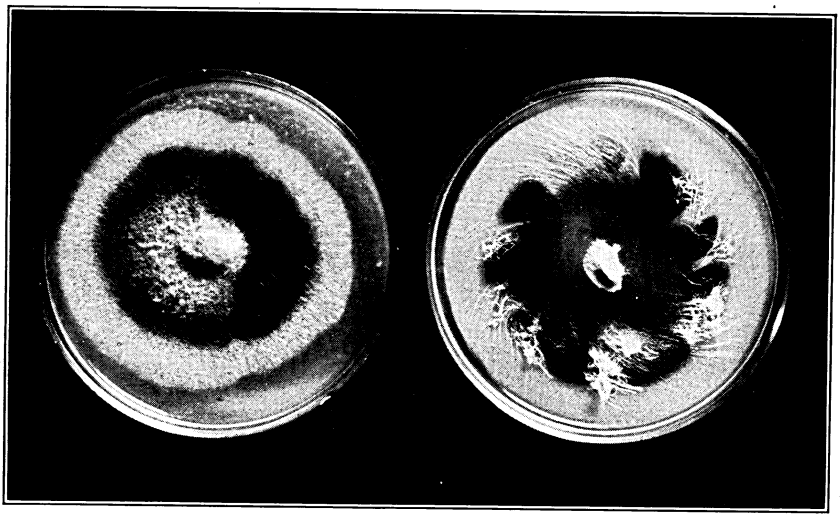

Fig. 4. Races 61 and 40 of *H. gramineum* on Potato-Dextrose Agar
The light portion of race 61, on left, was reddish-orange and the dark portion was black. The "paddle wheel" type of growth distinguishes race 40, on the right.

Pigmentation.—DeHaan (5) and Isenbeck (16) observed differences in color of aerial mycelium and in pigmentation of the substratum between races of *H. gramineum*. The many contrasting shades of color observed both in the mycelium and in the substratum have added to the striking dissimilarity of many of the cultural races. Varying intensities of black (races 2 and 77), brown (races 55 and 58), red (races 7 and 34), and yellow (races 26 and 47) were observed. (See Table 2.) Repeated tests of certain races on the same type of medium under similar conditions indicate that pigment production was a definite racial characteristic. Similar tests with the same organism proved that the type of medium materially influenced color production. Thus races 4 and 66, grown on opposite sides of the same petri dishes, both produced black pigmentation on potato-dextrose agar but developed black and orange-red, respectively, on rice agar (Figure 5).

Table 2

Cultural Characteristics of 20 Races of *Helminthosporium gramineum* Grown on Potato-Dextrose Agar at Room Temperature

Race No.	Source	Type of growth	Color of mycelium	Color of substratum	Diameter of colonies in mm.
2	Minnesota	Cottony	Light grayish olive	Dark olive	75.00 ± 1.80
6	Minnesota	Felty to cottony	Light grayish olive to shrimp pink	Gray to black	75.67 ± 0.88
7	Minnesota	Cottony	Dark olive to cinnamon	Peach red	66.75 ± 0.50
17	Minnesota	Velvet	Dark olive to cinnamon	Dark olive to cinnamon	74.25 ± 0.84
22	Minnesota	Cottony to velvet	White to light grayish olive	Dark olive to brick red	69.50 ± 1.01
26	Minnesota	Appressed to cottony	Cinnamon brown to coral red	Yellow ochre to grayish olive	78.00 ± 1.29
27	Minnesota	Felty to velvet	Dark olive to light grayish olive	Rose pink to warm buff	54.50 ± 1.01
34	Minnesota	Felty to velvet	Light grayish olive to dark olive	Morocco red	84.25 ± 0.51
46	Minnesota	Appressed radial	Light grayish olive to peach red	Buff yellow to peach red	79.00 ± 0.67
47	Minnesota	Appressed	Light grayish olive to peach red	Buff yellow to shrimp pink	77.00 ± 0.91
50	Iowa	Matted to cottony	Light grayish olive to dark olive	Dark olive to brick red	78.00 ± 1.29
54	Kansas	Cottony to felty	Light grayish olive	Peach red to light grayish olive	51.75 ± 1.66
55	Nebraska	Cottony to felty	Light grayish olive to peach red	Brazil red to dark grayish brown	72.50 ± 1.12
58	Nebraska	Velvety to felty	Peach red to cinnamon brown	Peach red to dark grayish brown	48.50 ± 0.19
68	South Dakota	Cottony to felty	Light grayish olive to cinnamon brown	Morocco red	88.00 ± 1.03
70	South Dakota	Velvet	Light grayish olive to peach red	Peach red	69.00 ± 0.91
77	Wisconsin	Woolly to cottony	Dark olive	Light grayish olive to dark olive	69.00 ± 0.00
78	Wisconsin	Woolly	Dark olive	Light grayish olive to dark olive	70.25 ± 1.11
81	Wisconsin	Felty to cottony	Light grayish olive to peach red	Maroon to dark olive	59.33 ± 0.92
86	Germany	Cottony to felty	Light grayish olive to apricot orange	Brick red	45.33 ± 0.83

* Where more than one color or type of growth is given, the first one mentioned is predominant. Color description is made according to Ridgeway's color standards.

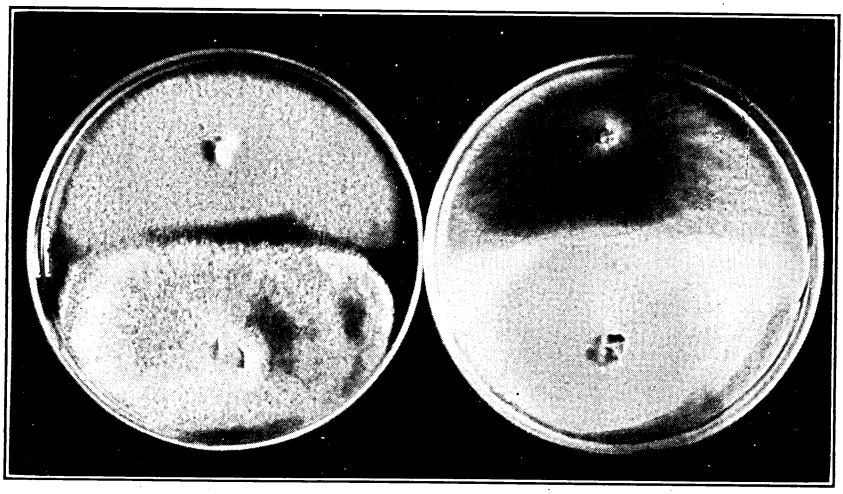

Fig. 5. Races 4 and 66 of *H. gramineum* Grown Side by Side in Petri Dishes on Two Different Media, Showing Effect of Medium on Color of Culture
Left, on potato-dextrose agar, both races gray-black. Right, on rice agar, race 4 black, and race 66 red orange.

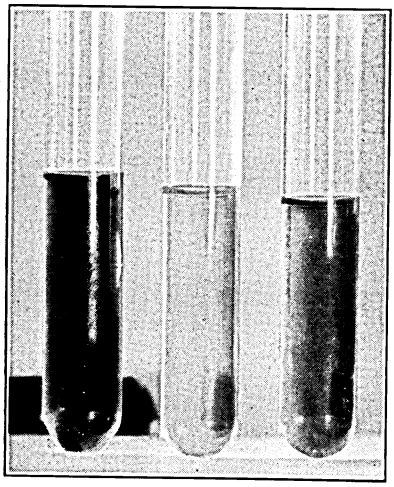

Fig. 6. Bacto-Peptone Broth Inoculated with Three Races of *H. gramineum*
After 4 weeks race 34 (left) turned the broth dark red-brown; race 2 (center) did not change the color of the broth, while race 22 (right) turned the broth light amber.

To study further the effect of nutrients on pigment production, five races were grown in 250 cc. Erlenmeyer flasks on the following four media: 2 per cent potato-dextrose agar, 2 per cent potato-glycerine agar, cornmeal agar, and agar plus extract from green barley leaves. The results are summarized in Table 3. As in previous tests, the type of medium influenced the color of mycelium and production of pigments in the substratum. The results substantiate those of previous tests and indicate that races may behave similarly on one medium, but decidedly different on another.

The pigment production of 30 races was studied also in bacto-peptone broth. The differences between the races in coloration of the liquid medium were even greater than on solid media. Some of the races changed the original straw-colored liquid medium to blackish brown,

others to dark red, while others produced no visible change (Figure 6). Many intergrading shades of color were produced. Determinations of pH were made on extracts from the 30 races by means of a quinhydrone electrode, but the change in pigmentation was not associated with a change in pH.[1]

Table 3

Differences in Pigment Production by Five Races of *Helminthosporium gramineum* on Four Different Solid Nutrient Media

Race No.	Potato-dextrose agar		Potato-glycerine agar		Cornmeal agar		Agar+extract from barley leaf	
	Mycelium	Substratum	Mycelium	Substratum	Mycelium	Substratum	Mycelium	Substratum
11	Dark gray	Dark gray	Gray	Gray	Dark gray	Gray to light red*	Dark gray	Gray to slightly red
14	Dark gray	Light red	Gray	Blackish	Gray	Light gray	Light red	Light red
22	Dirty gray	Yellow red to black	White	Yellow to black	Dark gray	Light red to black	Gray	Red to green
84	Light gray	Rusty	White	Yellow to white	Gray to slate	Colorless	Light gray	Lilac
85	Light gray	Yellow	Light gray	Yellow to black	Purple to red	Purple to red	Light gray	Slightly purple

* Where more than one color is given, the first one mentioned is predominant.

Association with bacteria.—Another means of distinguishing physiologic differences between races of *H. gramineum* was discovered. A bacterial contaminant in several petri dishes seemed to stimulate or induce pigment production by certain races but not by others. Therefore, the behavior of 12 races in association with a strain of bacteria was studied. Results indicated striking differences in production of red pigment by different races when grown with the bacteria on opposite sides of the same petri dish. Race 2, for example, produced a red pigment, while none developed in the absence of the bacterial colony. In races 9, 50, and 53 pigmentation was greatly intensified when cultures were grown in association with bacteria. Race 44, on the other hand, produced no pigment when grown either in association with the bacteria or alone.

While making the above tests it was observed that the stripe organism ceased to grow at varying distances from the bacterial colony, the distance depending on the particular race of *H. gramineum* concerned. For instance, the bacterial organism was much more antibiotic to races 9 and 80 than to races 35 and 60, while the effect on races 3 and 44 was intermediate. The results, altho preliminary, show that races of *H. gramineum* differ considerably in their physiologic relations to at least some other microorganisms.

[1] The writers are indebted to Dr. R. H. Landon for his help in determination of pH.

Rate of growth.—The relative rates of growth on nutrient agar differentiate certain races of *H. gramineum* very clearly.

Comparison was made of rates of growth of 85 cultural races on potato-dextrose agar in quadruplicate plates. The diameters of colonies were measured at the end of the fourth and eighth days. The data for 20 representative races, given in Table 2, show that there were statistically significant differences in the diameters of colonies (Table 4). For instance, the mean diameter of colonies of race 58 was 48.50 ± 0.19 mm. while that of race 68 was 88.00 ± 1.03 mm., a difference of 39.50 mm. It is obvious from Table 4 that a difference of only 4 or 5 mm. is usually statistically significant. For instance, the difference between races 17 and 77 was 4.75 ± 0.84 mm., which is distinctly significant.

Table 4

Summary of Statistical Differences in Growth Rates of 14 Races of *Helminthosporium gramineum* Grown on Potato-Dextrose Agar (based on figures given in Table 2)

Races compared	Difference between means, in microns	Difference between means divided by probable error of the difference
2 and 47	2.00 ± 2.02*	0.99
2 and 50	4.00 ± 1.17	3.42
6 and 46	3.33 ± 1.11	3.00
7 and 17	7.50 ± 0.98	7.65
7 and 81	7.42 ± 1.04	2.16
17 and 77	4.75 ± 0.84	5.65
27 and 58	6.00 ± 1.03	5.82
27 and 81	4.83 ± 1.37	3.53
68 and 78	17.75 ± 1.51	11.75

* Most of these comparisons were selected on account of their relatively small differences.

The average daily growth of colonies of 85 different cultural races was determined also from the fourth to the eighth day and is shown graphically in Figure 7. In the curve the races are grouped according to the nearest whole number representing their daily growth in mm. The daily growth actually varied from 2.94 ± .10 mm. for race 16 to 8.75 ± .08 mm. for race 34, a difference of 5.81 ± .04 mm. Both races were isolated from diseased plants collected at University Farm.

Effects of Ultra-Violet Irradiation

Stevens (22), Greaney and Machacek (12), and others have shown that ultra-violet irradiation may have profound effects on growth, fructification, mutation, and other characters in certain fungi. The writers therefore made attempts to determine whether races of *H. gramineum* would react differently to ultra-violet light. A Cooper Hewitt mercury vapor lamp operating on an alternating current carrying 125 volts was used. The races tested were grown on standard potato-

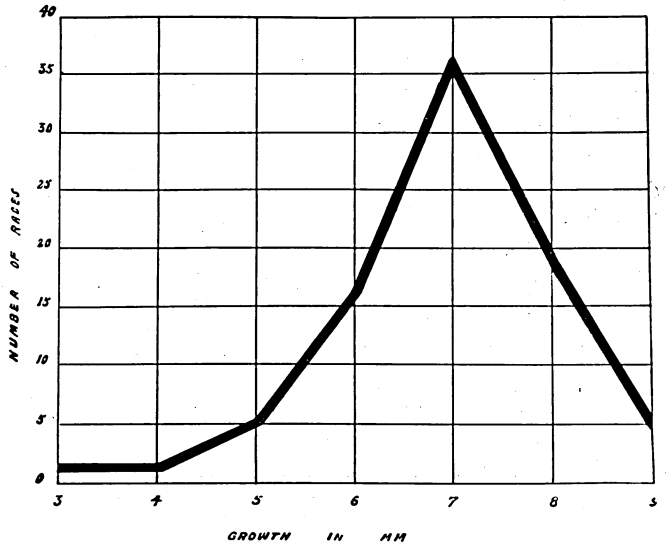

Fig. 7. The Relative Daily Rate of Growth of 85 Races of *H. gramineum* as Determined by the Growth from the Fourth to the Eighth Day of Quadruplicate Colonies Growing on Potato-Dextrose Agar

dextrose agar in 9 cm. petri dishes with the covers removed and exposed to the light at a distance of approximately 9 cm. The first exposures were made when the cultures were three days old, and they were repeated on four successive days. There was a marked retardation in the rate of growth of the colonies exposed to ultra-violet rays, but no other change was apparent. These treatments did not stimulate production of perithecia or conidia nor induce sectoring. However, striking differences in tolerance to the light were observed between cultural races 102 and 103, the former being more tolerant than the latter. This difference held true when the experiment was repeated at a later date. The percentage of retardation was based on the growth of the checks. With a one-minute exposure, the growth of race 102 was retarded approximately 56 per cent daily and with a two-minute exposure, the retardation was 80 per cent, while the growth of race 103 was checked 75 per cent with a one-minute exposure and about 78 per cent with a two-minute exposure. Apparently a daily exposure of two minutes was too severe for proper differentiation between the two races. These preliminary tests of differences in tolerance to irradiation between races led to additional studies, in which a more powerful carbon arc light was used. This light operated on an alternating current carrying 110 volts and 78 amperes. The "sunshine U" carbons were used to form the arc. In this test six races of *H. gramineum* were irradiated. After two days' growth on cornmeal agar the colonies

were irradiated two minutes, followed by a similar exposure 24 hours later. The covers were removed from the petri dishes which were then held in a vertical position 72 cm. from the source of light. Measurements of quadruplicate colonies were made for four successive days after the first treatment. In Table 5 is given a summary of the results obtained. The growth of all races was decidedly checked, the retardation being 64 per cent or more when compared with check colonies. As in previous tests, the different races reacted differently to irradiation. For example, race 97 was retarded 86 per cent in comparison with the check, while race 52 was retarded 64 per cent in comparison with the check, a difference of 22 per cent. On the other hand, races 88 and 97 behaved more nearly alike, the retardation being 82 and 86 per cent respectively, or a difference of 4 per cent. The effect on races 3 and 102, however, was about the same as compared with their respective checks. Examination of Table 5 will also reveal that there is no association of the rate of growth and the degree of retardation after treatment.

Statistical analysis of these data indicates that the differences are significant for some races but not for others. Six of the seven comparisons involving five races and listed in Table 6 were mathematically

Table 5

Effects of Irradiating with a Carbon Arc Ultra-Violet Light on the Rate of Growth of Six Races of *Helminthosporium gramineum*

Race	Treated	Untreated	Growth of treated in per cent of check	Inhibition in growth in per cent of check
3	12.7*	47.0*	27	73.67 ± 0.89
52	16.2	45.0	36	64.67 ± 0.56
66	4.5	14.0	32	68.50 ± 3.84
88	4.5	25.0	18	82.25 ± 0.89
97	7.5	53.0	14	86.00 ± 2.54
102	8.8	31.5	27	73.00 ± 2.10

* Total growth in mm. after 4 days on cornmeal agar. Average of four plates.

Table 6

Statistical Summary of Differences in Retardation of Growth of Five Races of *Helminthosporium gramineum* after Exposure to Ultra-Violet Light

Races	Difference between means and probable error	Difference between means divided by probable error of the difference
3 and 52	9.0 ± 1.05*	8.57
3 and 88	8.59 ± 1.26	6.81
3 and 97	13.33 ± 2.69	4.95
52 and 88	17.69 ± 1.05	16.84
52 and 102	8.33 ± 2.17	3.83
88 and 97	4.75 ± 2.69	1.76
97 and 102	14.0 ± 3.30	4.24

* Most of these comparisons were selected on account of their relatively small differences.

significant. It is clear therefore that some races of *H. gramineum* react differently to ultra-violet irradiation.

Morphological Differences in Conidia

Christensen (2), Henry (15), Greaney and Bailey (11), and Mitra (18) found considerable differences in length, width, and number of septa of conidia produced by races of certain species of *Helminthosporium*. Ravn (19), Drechsler (8), and others reported considerable variability in morphology of the conidia of *H. gramineum*. Consequently, it seemed likely that there might be differences in size and in number of septa or conidia of different races. Accordingly, a comparison was made of conidia from four parasitic races. The spores were produced on barley plants in the greenhouse. One hundred or more conidia of each race were measured to the nearest micron by means of an eyepiece micrometer. There were marked differences in length of conidia between the four races (Tables 7 and 8). The mean lengths of the conidia of races 14, 42, 56, and 60 were 65.7 ± .74, 101.4 ± 1.48, 80.5 ± 0.21, and 90.8 ± 1.36 microns, respectively. The greatest variation in the mean length of conidia was between races 14 and 42, a difference of 35.7 ± 1.41 microns, while the smallest difference, 10.3 ± 1.21 microns, was observed when races 56 and 60 were compared (See Figure 8).

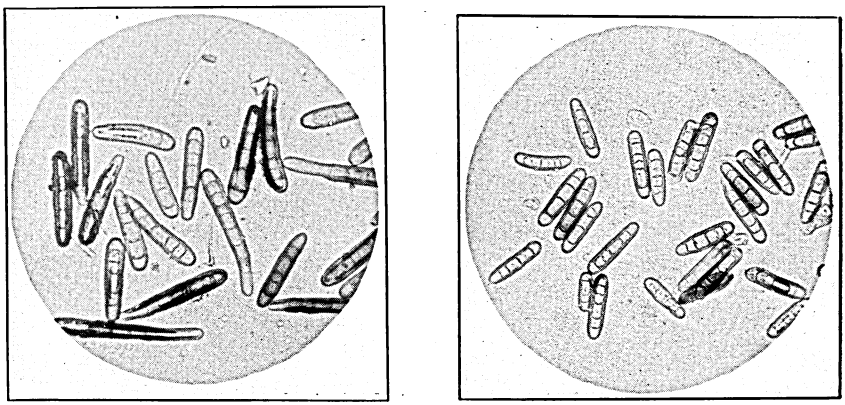

Fig. 8. Morphological Differences between Two Races of *H. gramineum*
Left, Race 42, 101.4 ± 0.21 × 24.7 ± 0.14 microns.
Right, Race 14, 65.7 ± 0.74 × 19.7 ± 1.25 microns.

The width of the conidia differed to some extent, altho the relative differences in measurements between the races were not nearly so great as the differences in measurements in the length. The mean width of the conidia of races 60 and 56 were 25.7 ± 0.28 and 19.7 ± 0.46 microns, respectively, the difference between the two being 6.0

± 0.27 microns. It is evident from Table 8 that all differences in length and width between conidia of the four races were statistically significant except the differences in width for races 14 and 56. In the four races the number of septa per conidium was more constant than width of conidia. Nevertheless, the differences in number of septa in conidia of the four races studied were statistically significant, except for races 42 and 56. The range in length and width of conidia measured was 30.4 × 15.2 microns to 167.2 × 26.6 microns. Race 14 had the least variability in length of conidia, a range of 53.2 microns, while those of race 42 varied over a range of 110.2 microns. The greatest variability in width of conidia was 10.4 microns; this was noted in race 14. If conidia from a greater number of races and from different sources had been measured, possibly much wider variation would have been encountered. It seems apparent from the above data that the size and variability of conidia depends to a considerable extent on the race concerned. It also seems evident that certain races of *H. gramineum* are morphologically as well as physiologically distinct. The measurements and number of septa of conidia of a single race may not necessarily coincide with published data for the species, but the averaged results obtained from the four races studied agree fairly closely. The average size of 427 conidia given in Table 7 is 83.6 × 22.5 microns and on the average there are three or four septa in each.

Table 7

The Relative Length, Width, and Number of Septa in Conidia of Four Races of *Helminthosporium gramineum* Taken from Barley Leaves

Race*	Number spores measured	Length in microns		Width in microns		Septa	
		Mean	Range	Mean	Range	Mean	Range
14	103	65.7 ± 0.74	30.4 — 83.6	19.7 ± 1.25	15.2 — 26.6	3.2 ± 0.07	1 — 5
42	112	101.4 ± 1.48	57.0 — 167.2	24.7 ± 0.14	19.0 — 26.6	3.6 ± 0.06	2 — 7
56	100	80.5 ± 0.21	45.6 — 106.4	19.7 ± 0.46	15.2 — 26.6	3.6 ± 0.02	2 — 6
60	112	90.8 ± 1.36	45.6 — 152.0	25.7 ± 0.28	22.8 — 26.6	3.9 ± 0.03	1 — 7

* Races 14 and 42 are from Minnesota, race 56 from Nebraska, and race 60 from Missouri.

Table 8

Summary of Statistical Differences in Relative Length, Width, and Number of Septa in Conidia of Four Races of *Helminthosporium gramineum*

Races compared	Difference			Differences between means divided by probable error of differences		
	Length*	Width*	No. of septa	Length	Width	No. of septa
14 and 42	35.7 ± 1.41	5.0 ± 1.11	0.4 ± 0.11	25.3	4.5	3.6
14 and 56	14.8 ± 0.31	0.0 ± 1.33	0.4 ± 0.02	47.0	0.0	13.7
14 and 60	25.1 ± 1.30	6.0 ± 1.20	0.7 ± 0.02	19.3	5.0	35.0
42 and 56	20.9 ± 1.30	5.0 ± 0.24	0.0 ± 0.06	16.0	20.8	0.0
42 and 60	10.6 ± 1.60	1.1 ± 0.20	0.3 ± 0.03	6.6	5.4	10.0
56 and 60	10.3 ± 1.21	6.0 ± 0.27	0.3 ± 0.02	8.5	22.5	15.0

* In microns.

Variation in Cultures

Helminthosporium gramineum up to the present time has been considered a rather stable species (8, 16) in contrast to *H. sativum* P. K. & B., a very unstable species (3). In fact, sectoring, or so-called mutation or saltation, has never been reported for the former. The present studies indicate that there are an indefinite number of cultural races which are widely distributed in nature. These results suggest that new races are arising either by hybridization, mutation, or as a result of heterocaryosis. Since the perfect stage has never been found by the writers, altho a careful search has been made, and also because it rarely has been found in nature, it seems logical to conclude that hybridization does not play a very important rôle in production of new races. In the present study, however, sectors and "patch" variants both occurred frequently in colonies of certain races on artificial media.

Helminthosporium gramineum, however, does not appear to sector as readily as *H. sativum* on nutrient media (3). In one test of 85 races grown on potato-dextrose agar in quadruplicate, in petri dishes, only a few races developed one or more sectors. In another test, in which 65 newly isolated monosporous cultures were tested, 10 per cent of them gave rise to sectors. Most of the races studied have not produced sectors, altho some have sectored rather frequently, as was the case in races 61 and 130 (Figures 4 and 9).

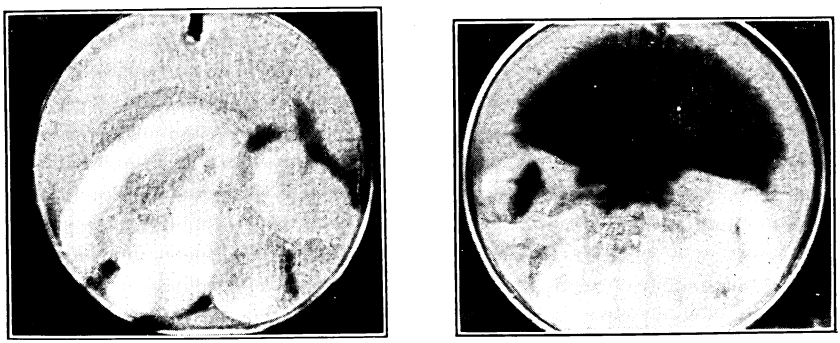

Fig. 9. Race 130 on Potato-Dextrose Agar Showing Sector-Like Growth
Left, top view; right, bottom view; the lighter part is the variant.

Many of the sectors were sharply delimited from the parent culture by type and rate of growth and by pigmentation. A number of these sectors were isolated and their cultural characteristics compared with their parents and other known races. Differences between the parents and variants were fully as well marked as those differences between races isolated from different states or even widely separated countries. Figure

10 illustrates differences between the parent colony (race 61), which is reddish orange in color and with little aerial mycelium, and a black variant (61-1) with fluffy aerial mycelium. Table 9 summarizes the conspicuous differences between 8 variants and their parents. In Figure 11 are presented races 101, 128, 129 and the variant of each. The dense aerial mycelium of the parent race 101 is strikingly different from its variant 101-1 which has such a sparse and appressed mycelium that it is scarcely visible in the photograph, altho its growth covers more than one half of the petri dish. The cultural differences between the other two races and their respective variants are obvious. These variants appear to be the result of genotypic changes, because they persisted through a number of cultural generations under different conditions.

Fig. 10. Variant 61-1 and Its Parent (lower half of plate), Showing Differences on Potato-Dextrose Agar

Another type of variant which was observed rather frequently was that which arose as distinct "patches" in the colonies (Figures 3 and 12). Such "patch" variants appeared to be characteristic of certain races only. It should be distinctly understood, however, that all "patch" formations are not true variants. A number of "patch" variants were isolated and compared in the usual manner with their parents in 250 cc. Erlenmeyer flasks containing 25 cc. of nutrient media. The variants in nearly every case appeared to be as distinct culturally from their respective parents as those which arose as sectors and have persisted through several cultural generations.

Most of the variants isolated appeared to be as stable as their original parents, altho several variants again sectored or gave rise to other "patch" variants. On one occasion a variant gave rise to another sector while still on the original plate.

The production of variants in culture of *H. gramineum* is rather significant, and may be indicative of what actually occurs in nature. Since at least two different parasitic races (60 and 61) were isolated from the same portion of a leaf, it seemed probable that new races of *H. gramineum* might arise in the host. Therefore, to determine if variants did arise in the host, Svansota barley was inoculated singly with monosporous races 14, 42, and 56, three very distinct cultural races. When the infected plants were in the jointing stage they were placed in

Table 9

Comparative Cultural Characters of Eight Races of *Helminthosporium gramineum* and a variant from each

Races and variant	Amount of growth*	Type of growth	Color of mycelium	Color of medium
16	36	Slow, irregular, felty	Dull reddish orange	Reddish orange
16-1	22	Slow, irregular, felty	Very dull pinkish orange	Dull pinkish orange
123	79	Fine, smooth, cottony	White to faint olive gray	Patches of purplish red
123-1	61	Felty	Dull purplish red	Orange to deep reddish maroon
124	29	Irregular, cottony	White	Faint reddish orange to white
124-1	42	Raised center, cottony	White to faint pink	Center, reddish orange; margin, white
125	60	Cottony, distinct white band at margin	White to very dull purple	Brilliant reddish maroon
125-1	41	Very fine cottony	White	White
126	73	Distinct felty	White to olive gray	Light grayish olive to dark grayish olive
126-1	15	Cottony	White to pale orange	White to deep reddish orange
127	60	Cottony	White to light grayish olive	White to grayish olive
127-1	57	Raised center, felty	Dull purplish maroon	Orange to deep reddish maroon
128	53	Very fine, cottony	Pinkish orange	Orange to pale maroon
128-1	70	Very fine felty	Orange to deep maroon	Orange to maroon
129	64	Cottony to felty	White to orange red	White to deep orange red
129-1	57	Fine felty	Deep dull maroon	Deep maroon

* Growth in mm. after 12 days on potato-dextrose agar. Average of two plates.

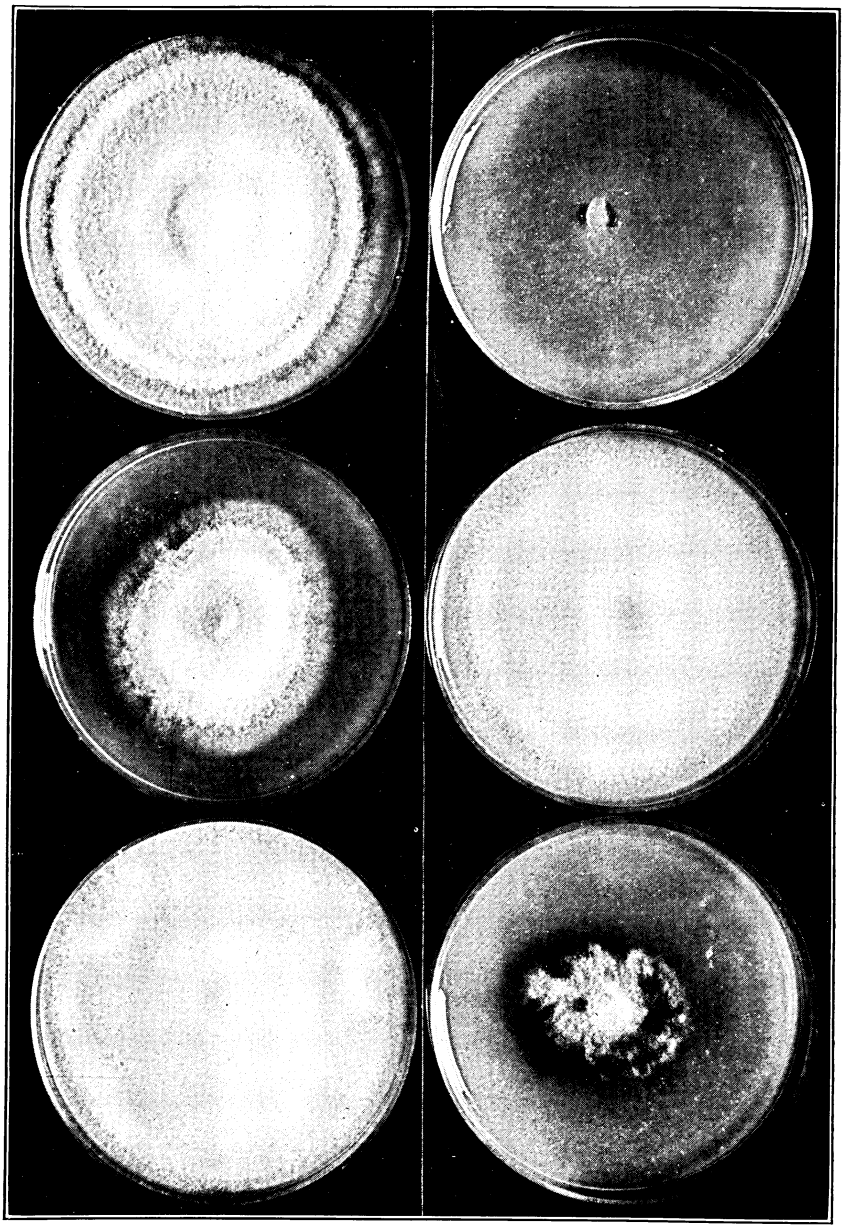

Fig. 11. Colonies of 3 Races of *H. gramineum* and a Variant from Each
Left row, top to bottom,—(parents) Races 101, 128, and 129.
Right row, top to bottom,—(variants) Races 101-1, 128-1, and 129-1.
Note the sparse and appressed growth of the variant 101-1 nearly covers the agar surface.

separate moist chambers in order to induce conidial fructification. About 100 conidia of each race were isolated. The isolates were then compared in Erlenmeyer flasks containing potato-dextrose agar. The results are presented in Table 10.

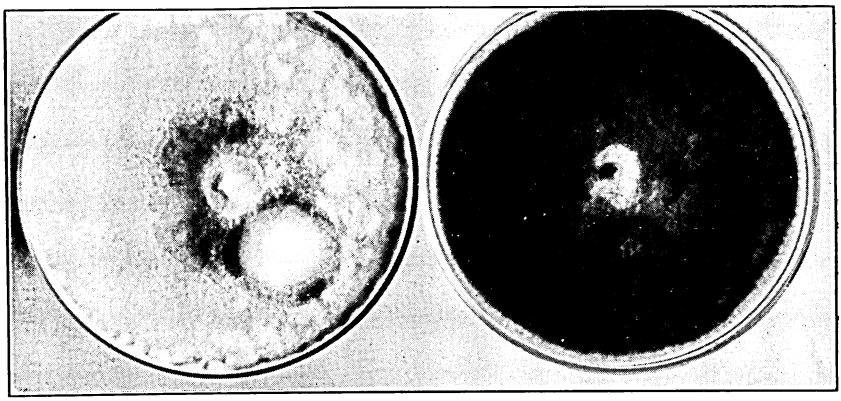

Fig. 12. Variation in Color of Two Races of *H. gramineum* Isolated from the Same Leaf of Barley Growing on Potato-Dextrose Agar

Left, race 60, white; right, race 61 which has red radical strands with a white center. Note distinct "patch" variant in race 60.

Of the 108 isolates obtained from the host inoculated with race 56, all developed identical colonies and were indistinguishable from the original parent. The monosporous progeny derived from race 14 produced three distinct types of colonies; 86 of 92 isolates, however, were alike. There was a tendency among many of the isolates to produce tufts and "patch-like" variants, but not distinct sectors (race 21, Figure 3). But the 84 isolates from the host inoculated with race 42 proved variable. Nine different cultural groups could be recognized easily, the largest group containing 35 isolates. Four of the isolates were placed in the tenth group because they developed so many sectors that proper grouping was impossible. There also was a tendency for many of the monosporous isolates to produce tufts or patches of aerial mycelium. Representative isolates from each group were tested the second time on a cultural medium. The results were in accord with the first grouping.

Two of the new isolates, 42-h31 and 42-h40 obtained from reinoculation of the host were passed back to the living barley plants. In the next conidial generation 85 and 48 monosporous isolates, respectively, were compared in duplicate in Erlenmeyer flasks. The progeny of 42-h81 and 42-h80 fell into three cultural groups. In this test 36 of the 85 isolates from 42-h80 or 42 per cent developed one or more sectors, while the progeny of 42-h80 produced no distinct sectors.

Table 10

The Variation in Cultures Obtained by Monosporous Isolation from Barley Infected with Three Different Cultural Races and Two Sub-races of *Helminthosporium gramineum*

Race	Number of isolates	Number of groups	Frequency in groups	Tendency to sector or produce "patches"
14	92	3	1, 5, 86	slight
56	108	1	108	very slight
42	84	10	1, 2, 2, 3, 4, 4, 4, 14, 15, 35	very great
42-h31*	85	3	13, 38, 51	very great
42-h80*	48	3	1, 7, 40	slight

* Races 42-h31 and 42-h80 were selected from the cultures obtained from barley infected by race 42, the former from an unstable group characterized by small tufts or "patches," the latter from a group that appeared stable.

These results tend to prove that certain races of *H. gramineum* are more stable than others and that certain races may continue to split up into new genotypes for at least three conidial generations. The results also indicate that at least nine distinct cultural races can be obtained from a single race after passing it back to the host. It is possible that many more races might have been isolated had a large population been selected or had different races been studied.

The exact method by which these variants arose was not determined. Hansen and Smith (14) accounted for the variation in *Botrytis cinerea* on the basis of heterocaryosis. Dickinson (7) in his studies on the nature of saltation found that the conidia of *Helminthosporium spp.* originated from a single nucleus. Therefore, it seems unlikely that heterocaryosis can account for the numerous variants of *H. gramineum,* the variants probably being mutants.

Studies of Pathogenicity

Isenbeck (16) found that seedling inoculation, either with spores or with mycelium, was as reliable an index of varietal susceptibility or resistance as floral inoculations with spores. This fact is of considerable importance in making pathogenic studies.

Greenhouse studies.—In order to obtain preliminary information on the parasitic behavior of 75 cultural races of *H. gramineum,* pathogenicity studies were made in the greenhouse. Cultures of the different races were grown on potato-dextrose agar in petri dishes. After about 12 days' growth the mycelium was chopped up and mixed with disinfected barley seed. About 15 cc. of water was added to each petri dish. Seven varieties of barley were inoculated and incubated at 15 degrees C. for seven days. Then the contents of the petri dishes were

Table 11

Result of Inoculating Seven Varieties of Barley with 24 Cultural Races of *Helminthosporium gramineum* in the Greenhouse in 1931

Race	Source	Varieties and percentages of infection						
		Black Hull-less	Glabron	Lion	Peatland	Trebi	Velvet	White Hull-less
2	Minnesota	5	..*	..	33	4	24	0
3	Minnesota	26	15	16	26	4	30	0
4	Minnesota	5	2	0	12	0	8	..
8	Minnesota	7	8	4	76	18	21	..
11	Minnesota	20	6	2	40	24	21	3
13	Minnesota	0	7	2	23	2	8	10
17	Minnesota	11	2	25	8	0	25	..
18	Minnesota	4	4	2	26	6	8	0
21	Minnesota	5	4	15	37	6	13	10
23	Minnesota	0	0	5	48	9	0	..
25	Minnesota	0	11	17	18	11	2	5
26	Minnesota	6	5	12	32	9	0	..
29	Minnesota	0	0	0	26	4	0	0
39	Minnesota	4	0	26	26	13	0	0
42	Minnesota	11	2	12	19	0	21	0
45	Minnesota	10	8	0	27	14	9	0
57	Nebraska	0	0	0	9	0	4	4
61	Missouri	0	13	6	8	11	0	..
65	South Dakota	9	..	32	75	33	0	0
66	South Dakota	2	0	0	30	14	18	..
67	South Dakota	14	..	..	38	15	10	12
72	South Dakota	17	4	8	30	0	15	..
74	South Dakota	9	7	19	17	8	0	4
86	Germany	0	0	0	9	0	0	0
Check		0	0	0	0	0	0	0

* Insufficient number of plants.

placed in four- or six-inch pots filled with steamed soil. The pots were placed in a greenhouse with temperatures varying from 10 to 20 degrees C. for about a week. After that the temperature was raised to between 20 and 28 degrees C. Notes were taken when the plants were two months old. Since the percentage of infection in most cases was not especially high, the behavior of only 24 representative races is given (Table 11). The percentage of infection varied considerably. For example, on Peatland the infection varied from 0 per cent for race 36 to 77 per cent for race 8 (Figure 13). Altho infection percentages were not so high on the other six varieties as on Peatland, the variability of percentage infection indicates sharp differences in parasitic capabilities among races. The results indicate also that certain races have a much wider range of parasitism than others. For example, race 86 attacked only Peatland, race 45 caused infection on five of the seven varieties, while race 21 produced infection on all seven varieties inoculated. In general, the greenhouse test indicated clearly that many of the races which were different culturally differed in parasitism also.

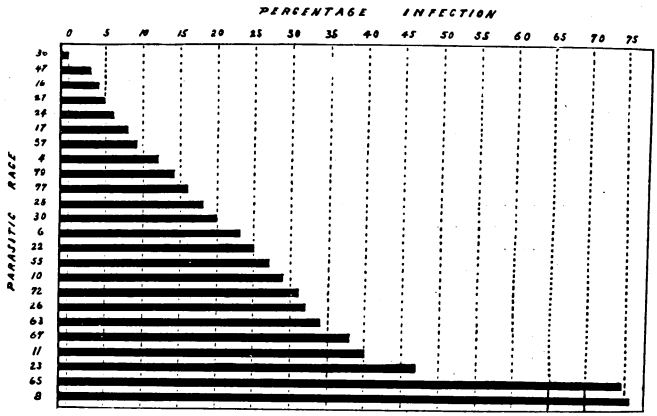

Fig. 13. The Relative Virulence of 25 Races of *H. gramineum* on Peatland Barley Grown in the Greenhouse, 1931

Field tests.—In 1931 the parasitic behavior of 12 cultural races of *H. gramineum* was tested on 16 varieties of barley in the field. These races were selected chiefly because of their marked differences in cultural characters. Five were from Minnesota (races 14, 21, 33, 34, and 42), three from Iowa (races 51, 52, and 54), two from Missouri (races 60 and 61), one from Nebraska (race 56), and one from Wisconsin (race 78). In order to inoculate the plants, seeds of the different barley varieties were planted in shallow wooden flats containing a mixture of steamed muck and sandy loam. The inoculum was increased in Erlenmeyer flasks containing a mixture of wheat and oats. All the varieties of barley tested to a given race were sown in the same flat, so as to expose them as far as possible to the same environmental conditions. For 10 days the flats were kept in controlled temperature chambers at 10 degrees C. During this period, however, three different times the temperature was raised to 30 degrees C. for approximately 24 hours. The plants were then transplanted into the fields in four-foot rows one foot apart. The number of plants varied from 50 to 100, usually 80 to 90. The final notes were taken when the plants were two months old.

It is obvious from Table 12 and Figures 14 and 15 that there were marked differences in the pathogenicity of the 12 cultural races. For example, race 34 produced 67 per cent infection on Glabron and 26 per cent on Trebi, while race 51 produced no infection on these two varieties. Race 14 developed 92 per cent infection on Svansota, 51 per cent on Velvet, and none on Himalaya, in contrast to race 34, which produced 60 per cent, 91 per cent, and 65 per cent infection, respectively. It is a significant fact that a given variety may be completely susceptible to

one race, but immune from or highly resistant to another (Figures 14 and 15).

Table 12

The Results of Inoculating 16 Varieties of Barley with Mycelial Cultures of 12 Races of *Helminthosporium gramineum* 1931

Variety and number	Cultural race and percentage of infection*												
	Check	14	21	33	34	42	51	52	54	56	60	61	78	Av.
Alpha C. I. 959.....	0	24	45	44	67	49	10	13	0	68	0	57	10	32.2
Black Hull-less (Selection)	0	5	3	9	11	6	0	0	0	0	0	2	2	3.1
Coast, C. I. 690.....	0	23	46	44	59	45	9	3	26	11	7	17	0	24.1
Colsess, Minn. 461.....	0	34	30	44	63	55	6	24	7	33	28	43	2	30.7
Glabron, Minn. 445.....	0	27	3	50	67	35	3	0	0	18	0	33	2	19.8
Himalaya, C. I. 2257.....	0	0	31	0	65	0	0	0	22	17	7	33	0	14.5
Lion, Selection	0	10	40	38	5	16	9	0	9	17	10	45	4	16.9
Manchuria, Minn. 184.....	0	50	67	50	82	70	8	35	12	22	25	48	19	40.6
Peatland, Minn. 452.....	0	40	8	69	38	69	9	2	30	35	31	57	6	32.8
Peru, C. I. 2302.....	0	45	39	32	65	49	15	0	9	26	24	50	14	30.6
Svansota, Minn. 440.....	0	92	55	60	60	58	31	8	23	35	46	48	23	44.9
Trebi, Minn. 448.....	0	0	6	0	26	2	0	2	14	11	14	8	5	7.3
Velvet, Minn. 447.....	0	51	33	65	91	74	15	0	0	35	0	39	14	34.7
White Hull-less (Selection)	0	19	6	35	36	9	2	0	9	19	10	12	0	13.0
Odessa, Selection	0	21	27	46	65	48	2	2	0.2	16	16	33	0	23.0
——— C. I. 694.....	0	0.3	4	9	0.1	14	0	0	0	3	0	11	3	3.6
Average.....		27.5	27.7	37.1	50	37.5	7.4	5.5	10.0	22.9	13.6	33.5	6.5	

* Races 14, 21, 23, 34, 42 from Minnesota; races 51, 52, 54 from Iowa; race 56 from Nebraska; race 60, 61 from Missouri; and race 78 from Wisconsin.

The data in Table 12 tend to prove that eight or more of the 12 races used in the field test were distinctly different in their parasitic capabilities. Their behavior on six varieties is presented graphically in Figure 15. Races 33 and 42, 54 and 60, 56 and 61, and 52 and 78, respectively, reacted somewhat similarly on the twelve hosts inoculated. Four of the

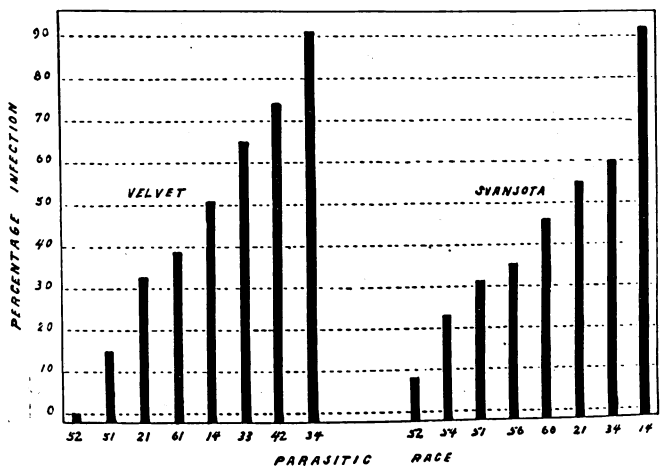

Fig. 14. The Relative Virulence of 8 Races of *H. gramineum* on Velvet and Svansota Barley, Respectively, Grown in Field, 1931

five races from Minnesota were distinctly different in their pathogenicity, while two were probably alike. It seems interesting and significant that races 60 and 61 isolated from the same portion of leaf collected in Missouri were distinctly different in parasitic capabilities.

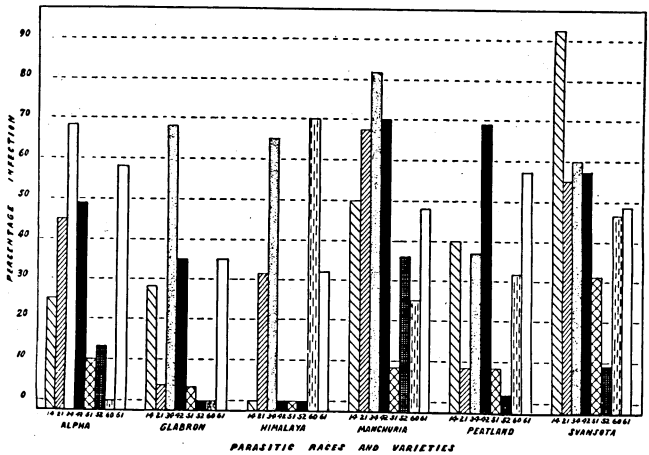

Fig. 15. The Relative Virulence of 8 Parasitic Races of *H. gramineum* on 6 Varieties of Barley Grown in the Field, 1931

It is also evident from Table 12 that some races were very virulent, some moderately so, and others relatively weak (Figure 16). Races 42 and 21 attack all the varieties to a greater or less degree, while race 52 was able to attack only 8 of 16 varieties. In considering the general virulence of a given race, all varieties tested, one finds a range of average infection from 5.5 per cent with race 52, to 50 per cent with race 34.

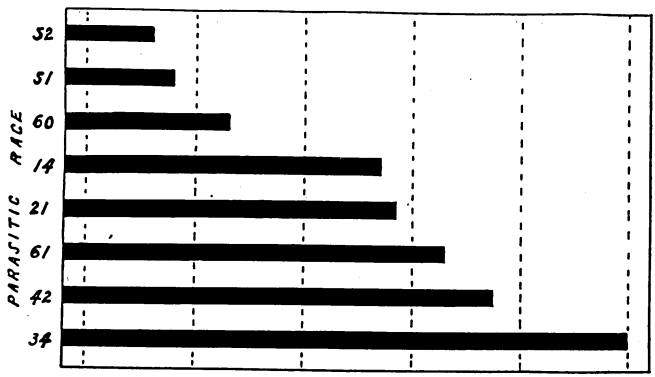

Fig. 16. The Relative Virulence of 8 Parasitic Races of *H. gramineum* on 16 Varieties of Barley (1931)

Field experiments similar to those carried out in 1931 were made in 1932 also. In this test 27 races of the organism were used along with 12 varieties of barley. Thirteen of the races used were selected from the group of cultural races isolated in 1931. The other 14 races were isolated from collections of stripe in Minnesota in 1932. The varieties were inoculated and transplanted to the field as in previous tests. Notes were taken when the plants were six weeks old. The total number of plants for each reading was usually 75, but varied from 60 to 100.

The data in Table 13 indicate the existence of numerous parasitic races of *H. gramineum.* As in previous tests, the 27 races differed greatly in ability to attack varieties of barley. However, percentages of infection in general were lower than those obtained in the field studies of 1931. This difference in infection in two years is probably due to variation in temperature during the incubation period. Nevertheless, at least 12 more parasitic races can be recognized by their differential effect on the 16 varieties of barley. If there had been a relatively higher percentage of infection, it might have been possible to recognize many more different parasitic races.

The variations among the three races of *H. gramineum* listed in Table 14 are consistent for the three replicates, altho percentages of infection are small. The data in Table 14 were analyzed by Fisher's analysis of variance method (9). In this study an attempt was made to ascertain whether the variation due to races, varieties of barley, and interaction of varieties $\times$ races was greater than that due to error. The significance of these factors was determined by Fisher's Z. It is apparent from Table 15 that the varieties are significantly different in their reaction to *H. gramineum,* while the relative virulence of the races as determined by the average infection on all varieties is not different. But the interaction of the varieties of barley $\times$ races of stripe is highly significant, since the value of Z exceeds the one per cent point. Thus it may be concluded that the different varieties behaved in a differential manner when inoculated with the three races. The standard error of the difference is 2.68, and two times this number, or 5.36, gives the minimum for statistical significance between any two means. This analysis indicates that the three races 74, 84, and 122 listed in Table 14 are significantly different in their parasitic capabilities.

If one compares the relative parasitic behavior of races 4, 34, and 60 on barley tested in two successive years, it is apparent that in general their parasitism was similar, the reaction of Glabron to race 34 being an exception. On the other hand, race 61 produced no infection on Glabron, Lion, and Velvet, altho in previous tests in 1931 these varieties appeared to be susceptible to this race. This discrepancy in the para-

Table 13

Results of Inoculating 12 Varieties of Barley with 27 Cultural Races of *Helminthosporium gramineum*, 1932

Race	Source	Varieties and percentage of infection												
		Black Hull-less	Glabron	Lion	Manchuria	Minn. No. 462	Minsturdi	Peatland	Spartan	Svansota	Trebi	Velvet	Wis. No. 38	Av.
2	Minnesota	4	7	17	16	11	32	37	0	32	4	11	5	9.0
12	Minnesota	0	0	0	..	4	4	0	0	0	0	0	2	0.9
14	Minnesota	0	24	0	..	17	25	50	0	28	0	30	14	17.0
34	Minnesota	5	31	0	..	16	51	21	0	39	0	36	3	18.3
50	Iowa	0	0	0	0	0	0	11	1	8	0	0	0	1.6
60	Missouri	0	5	12	21	10	8	16	0	7	8	0	14	7.7
61	Missouri	2	0	0	..	2	2	79	0	14	3	0	9	10.0
63	South Dakota	0	12	0	..	0	9	11	0	8	0	8	3	5.6
74	South Dakota	0	0	4	15	0	0	57	0	13	0	1	8	8.1
75	North Dakota	0	25	0	16	18	27	38	0	48	0	24	12	17.3
77	Wisconsin	0	4	0	10	0	0	21	2	4	2	22	14	6.5
80	Wisconsin	0	9	0	0	0	15	0	0	6	0	19	3	4.3
84	Canada	0	21	0	20	16	12	35	0	18	7	39	22	15.8
88	Washington, D. C.	0	0	0	0	0	0	0	0	2	0	0	0	0.1
89	Minnesota	0	0	14	32	12	43	50	0	30	0	32	23	19.6
90	Minnesota	.. *	7	2	25	10	10	6	0	10	0	7	0	6.4
91	Minnesota	0	0	0	0	0	0	3	0	0	0	0	0	0.2
92	Minnesota	5	16	0	9	14	16	32	0	35	0	32	0	13.2
93	Minnesota	0	0	0	0	0	1	0	0	0	0	9	0	0.8
94	Minnesota	5	24	1	29	17	17	40	3	20	5	14	9	16.1
95	Minnesota	0	0	0	3	0	4	0	0	0	0	0	0	0.5
96	Minnesota	0	0	0	0	0	0	0	0	0	0	18	14	2.6
97	Minnesota	0	19	0	31	3	44	33	0	27	0	22	0	14.9
98	Minnesota	0	7	0	6	6	11	24	0	15	0	15	7	7.5
99	Minnesota	0	0	0	0	2	6	0	0	2	0	0	0	0.8
100	Minnesota	6	0	0	11	0	3	67	3	38	4	..	9	12.8
101	Minnesota	0	0	0	2	0	0	10	0	9	0	8	0	2.4
Check		0	0	0	0	0	0	0	0	0	0	0	0	0.0
Average		1.0	7.7	1.8	11.2	5.8	12.6	23.7	0.3	15.2	0.9	13.9	5.4	

* Insufficient number of plants.

Table 14

The Variation in Percentage of Infection in Triplicate Plots When 12 Varieties of Barley Were Inoculated with One Race or with a Mixture of Races of *Helminthosporium gramineum*

Races or combination	Series	Varieties and percentage of infection											
		Colsess	Glabron	Lion	Manchuria	Minn. No. 457	Minn. No. 462	Minsturdi	Odessa	Peatland	Trebi	Velvet	Wis. No. 38
74	1	14	0	2	0	0	0	0	19	4	8	0	6
	2	9	0	7	0	0	0	0	29	12	2	0	4
	3	20	0	2	0	0	0	0	24	12	6	0	5
84	1	0	3	4	11	2	4	3	10	1	0	7	0
	2	0	7	0	17	2	5	7	10	2	0	13	0
	3	0	3	2	12	2	3	5	14	4	0	13	0
122	1	0	4	0	8	0	2	1	2	0	0	15	1
	2	0	10	0	9	2	5	3	3	2	0	30	2
	3	2	3	0	8	2	9	3	6	3	0	21	0
Combination of races 50, 60, 83, 84, 113 and 122; mass inoculations	1	2	4	6	4	4	0	0	9	0	4	14	0
	2	0	4	1	3	0	0	2	2	0	0	6	0
	3	0	1	2	5	2	0	3	2	0	0	16	0
Average of races 50, 60, 83, 84, 113, and 122; inoculated separately*	1	T**	1	1	5	T	1	2	3	T	0	4	T
	2	0	3	0	4	1	2	3	3	1	0	7	T
	3	1	1	T	4	1	2	2	3	1	2	6	0
Check	1, 2, 3	0	0	0	0	0	0	0	0	0	0	0	0

* Races were inoculated singly and then infection percentages were averaged.

** Trace, less than one per cent.

sitism of race 61 is probably due to a genotypic change in the organism. When race 61 was first isolated it sectored rather frequently. It is possible that different races of *H. gramineum* differ considerably in their parasitic capabilities under different environmental conditions. Johnson's (17) studies on the differential response of two races to temperatures adds support to this statement. It is also possible that different varieties respond differently to the same race under different conditions. This is known to be true for certain varieties of wheat in relation to the smut organisms, *Tilletia* spp. (1).

Table 15

The Analysis of Variance of the Stripe Infection Obtained by Inoculating 12 Varieties of Barley with Three Races of *Helminthosporium gramineum* when Grown in Triplicate Plots (See Table 14)

Variation due to:	Degree of freedom	Sum of squares	Variance	Z
Replicates	2	69.16	34.58	
Varieties of barley	11	1463.55	133.05	3.5965**
Races of stripe organism	2	22.16	11.08	
Interaction (varieties x races)	22	2372.95	107.86	3.4915**
Error	70	2762.82	39.47	
Total	107	6690.00		

** Value of *Z* exceeds 1 per cent point. S. E. of difference between two means = 5.13.

The writers realize that the significance of these results would be greater if replicate tests had been made. In 1932 two series of flats in duplicate were planted respectively with 10 varieties of barley inoculated with two combinations of different sets of three races of *H. gramineum*. On the ten varieties used, only two had a variation in infection greater than six per cent. There was a difference of 16 per cent infection between the series on Svansota and 11 per cent on Minn. No. 462. Furthermore, it should be remembered that, in the 1931 and 1932 studies, all varieties tested for parasitism by a given race were sown in a single flat and incubated in a large constant temperature room which seldom fluctuated more than 2 degrees C. The varieties, therefore, were exposed to approximately the same environment during the critical period for infection. The consistency of triplicate tests with a given race or group of races can perhaps best be gained by consulting Table 14. In these tests the method of inoculation and planting was the same as the field tests of 1931 and 1932. The variation in percentages of infection on the triplicates of a given variety was usually less than 5 per cent. Of the 180 possible comparisons, only four had differences in infection of 10 per cent or more. If the variety was susceptible or resistant in one replicate, it proved equally susceptible or resistant in the other two replicates.

It is obvious that two or more races which possess about the same degree of virulence on the varieties tested may or may not be distinct parasitic races. From a practical standpoint, slight variations, however, are of little importance. The results, especially those of 1931 and 1932, indicate quite clearly that *H. gramineum* comprises many distinct parasitic races. It seems reasonable to conclude from various inoculation tests that there are at least twenty, and very likely many more, distinct parasitic races of *H. gramineum*. It is likely that the use of more varieties would disclose even greater differences in parasitic behavior among the apparently similar races. Obviously, the existence of so many parasitic races is of great importance in the development of new varieties of barley.

In taking notes in the field and greenhouse, differences in the degree of infection were not recognized in tabulating the data presented here. A plant when examined was considered either diseased or healthy. In nearly every instance, when one culm was infected all culms of that particular plant were infected. However, certain differences in degree of infection were observed, but usually seemed to be due to varietal reactions rather than to the behavior of a given race of *H. gramineum*. For example, the lesions produced on the leaves of Trebi often were not so clear-cut as those on Peatland or Velvet. Stunting was much more pronounced in Svansota than in Minsturdi. There is considerable evidence, however, that the degree of stunting was influenced by the race involved. Weather conditions also seemed to be an important factor affecting the rate of development of the disease, as well as the severity of infection. Therefore, the amount of stunting and the degree of susceptibility were not used as criteria for distinguishing races of the stripe organism.

Combination of races.—Cultural studies have shown that several different races of *H. gramineum* may be isolated from the same plant. Therefore, studies on parasitism were made to determine the effect of inoculating with a mixture of races. In the greenhouse Velvet barley was inoculated with 18 different cultural races singly and also with 13 different paired combinations from the same races. Colsess barley was inoculated with 33 cultural races singly and with 25 different paired combinations. A total of 38 paired combinations were made. When two races were combined the average infection was 3 per cent. This was approximately the same as the average infection of the races inoculated singly, or 2.7 per cent. In four cases there was one per cent increase in virulence over the more pathogenic races, but never a decrease below the least pathogenic race.

A somewhat similar test was made in the field on 11 varieties of barley. A mixture of three races was used. The varieties also were inoculated separately with each race used in the combination tests.

The percentages of infection of the three sets of combinations agree fairly closely with average infection obtained when each of the three races was tested separately (See Table 16). One combination (races 2, 90, and 98), however, caused a higher percentage of infection than that obtained when these races were tested separately. In fact, a higher percentage of infection was obtained with this combination than with the most virulent of any of the single races. For instance, the combination of races produced 25 per cent infection on Glabron, 49 per cent on Svansota, while race 2, the most virulent race used, produced only 7 per cent and 32 per cent infection, respectively.

In 1933 a similar test was made in triplicate, using a combination of six races. The results are given in Table 14. The data was analyzed by Fisher's analysis of variance method (9), which is given in Table 17. The two methods were not significantly different, since value of Z does not reach the 5 per cent point. The value of Z for varieties exceeds the one per cent point, which indicates that the varieties differ in their reactions as determined from the average response to the two methods of inoculation. In this case the percentages of infection in three series agree fairly closely with the average infection of six races inoculated separately.

Table 17

The Analysis of Variance of Stripe Infection Obtained by Two Methods of Inoculation* with 6 Races of *Helminthosporium gramineum* on 12 Varieties of Barley when Grown in Triplicate Plots

Variation due to:	Degree of freedom	Sum of squares	Variance	Z
Replicates	2	8.78	4.39	.....
Varieties of barley	11	380.32	34.57	0.5807**
Methods*	1	11.68	11.68	0.0382
Interaction of methods × varieties	11	76.65	6.96	.....
Error	47	508.57	10.82	.....
Total	71	986.00	....	.....

* In one method the six races were mixed before inoculation, in the other each race was inoculated separately and the percentage of infection of six races averaged.

** Value of Z exceeds 1 per cent point. S. E. of difference between two means = 2.68.

Varietal resistance.—A number of workers have called attention to varietal resistance of barley to stripe, but no report has been made on the reaction of barley to a large number of parasitic races. Isenbeck (16) found that certain varieties behaved quite differently to three parasitic races of stripe organism. He also proved that seedling inoculation with mycelial cultures was as good a criterion of varietal resistence as in-

Table 16

Result of Inoculating 11 Varieties of Barley with Certain Races of *Helminthosporium gramineum* Alone and in Combinations of Threes

Races	Variety and percentage of infection										
	Black Hull-less	Glabron	Manchuria	Minn. No. 462	Minsturdi	Peatland	Spartan	Svansota	Trebi	Velvet	Wis. No. 38
Combination of 14, 74, and 84; mass inoculation	2	11	6	13	26	51	5	22	0	16	2
Average of 14, 74, and 84*	0	16	11	12	11	47	0	20	3	24	14
Combination of 61, 62, and 80; mass inoculation	0	4	0	2	2	38	0	5	0	4	4
Average of 61, 62, and 80*	1	7	9	1	8	30	0	9	1	8	7
Combination of 2, 90, and 98; mass inoculation	0	25	21	29	36	74	0	49	0	..	11
Average of 2, 90, and 98*	5	7	15	8	17	9	0	19	1	11	5
Combination of 92, 97, and 101; mass inoculation	0	0	7	11	5	0	0	11	0	12	25
Average of 92, 97, and 101	2	12	14	6	20	25	0	23	0	21	0
Check	0	0	0	0	0	0	0	0	0	0	0

* Races were inoculated singly and then infection percentages were averaged.

oculation with conidia either in the seedling or floral stage. Therefore, the present results on varietal resistence should be a fair index of varietal behavior to *H. gramineum*.

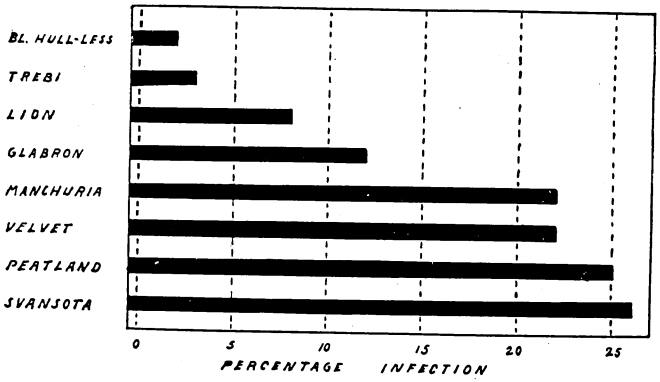

Fig. 17. The Relative Susceptibility of 8 Varieties of Barley to Stripe as Determined by Inoculation with 31 Races of *H. gramineum* (1931 and 1932).

The relative susceptibility of 17 varieties of barley to different races of the stripe organism used in the field studies of 1931 and 1932 is summarized in Tables 12, 13, and 14. The most heavily attacked varieties in 1931 were Peatland (33 per cent), Svansota (45 per cent), Velvet (35 per cent), and Manchuria (40 per cent). The most resistant ones were Trebi (7 per cent), C. I. 694 (4 per cent), and Black Hull-less (3 per cent). The data in Tables 12 and 13 indicate that varieties susceptible in 1931 were also susceptible in 1932. Minsturdi also was included in the latter test and proved to be susceptible, while Spartan was the most resistant. The average infections for eight varieties used in this investigation for two successive years, but not necessarily inoculated with the same races, are summarized in Table 18. In Figure 17 is presented graphically the behavior of 8 varieties to 31 different races of *H. gramineum*. In general, varieties that were usually susceptible under field conditions were most heavily and commonly attacked by the different races of stripe when artificially inoculated. However, some varieties were susceptible to some races but immune from, or highly resistant to, others. For example, Glabron, a high-yielding, smooth-awned variety, is generally much more resistant to stripe than Velvet, a sister selection, yet to certain races, such as 33 or 34, it is very susceptible. Even Black Hull-less, one of the most resistant varieties tested, was immune from, or highly resistant to, eight of 12 races of *H. gramineum* tested in 1931, yet developed 11 per cent infection when inoculated with race 34. Wisconsin No. 38 appears to be moderately resistant, but apparently fairly susceptible to certain races. It is obvious

that one should be cautious in making generalizations regarding the varietal resistance or susceptibility.

Table 18

The Relative Susceptibility of Eight Varieties of Barley Inoculated with 12 Races of *Helminthosporium gramineum* in 1931 and 27 Races in 1932

Variety and number	Percentage of infection		
	1931	1932	Average
Black Hull-less, Selection	3.1	1.0	2.1
Trebi, Minn. No. 448	7.3	0.9	4.1
Lion, C. I. 923	16.9	1.8	9.4
Glabron, Minn. No. 495	19.8	7.7	13.8
Manchuria, Minn. No. 184	40.6	11.2	25.9
Peatland, Minn. No. 452	32.8	23.7	28.8
Velvet, Minn. No. 457	34.7	13.9	29.3
Svansota, Minn. No. 440	44.9	15.2	30.1

DISCUSSION

Helminthosporium gramineum comprises an indefinite number of biotypes or races that differ from each other distinctly and consistently in pathogenicity and certain other physiologic characters, as well as in cultural characters on artificial media. These biotypes or collections of biotypes are, therefore, properly designated as physiologic races. There are distinct and statistically significant differences in the morphology of conidia of at least some of the races. This fact is supporting evidence that they differ genotypically.

Despite the very distinct differences between races, they have some essential characters in common. None of them sporulate on artificial media, altho they grow well on it. All of them produce typical stripe-disease symptoms on those varieties of barley to which they are pathogenic. While there are profound differences between some of the races, therefore, they resemble each other more than they resemble other similar groups of races, such as those of *Helminthosporium teres*, for example. Consequently, the writers are of the opinion that all should be considered as belonging to the species *H. gramineum*. The observed facts strengthen the growing attitude of mycologists, that a species is a concept and that many species comprise a group of biotypes that may differ distinctly in many clearly perceptible characters but yet have certain important and still more clearly perceptible characters in common.

It is increasingly clear, however, that the recognition of those characters that may suffice for taxonomic purposes do not suffice at all for pathologic requirements. That the pathologist and plant breeder cannot content himself only with the species is shown most emphatically by the results of the pathogenicity tests made by the writers. Certain

races of *H. gramineum* are relatively innocuous to certain varieties of barley but very pathogenic to others. To be sure of the reaction of a variety of barley to *H. gramineum*, it is necessary to expose the variety to infection by all the races that constitute the species, not merely to a few representative ones. In breeding new varieties this is doubly important, altho it is probable that resistance to several races may be governed by the same genetic factors. Furthermore, *H. gramineum*, like many other species of pathogenic fungi, is a dynamic phenomenon, not a static one. New biotypes apparently arise; hence the species comprises a population with changing complexion.

The writers observed the production or segregation of new biotypes through sectoring and "patch mutation" in colonies growing on artificial media. The genetic explanation for this variation has not been obtained. Neither is it known to what extent new biotypes are arising in nature nor how important they may be, either from the standpoint of taxonomy or pathology. Further investigation is highly desirable, as *H. gramineum* is not only a destructive pathogen but also a very suitable fungus, in many respects, for a basic study of fungus genetics.

SUMMARY

1. Approximately 1200 monosporous isolates of *Helminthosporium gramineum* were made from material obtained from 12 states of the United States and from Canada and Germany. Several hundred of these were studied in considerable detail.

2. The results indicate that *H. gramineum* comprises an indefinite number of races which differ from one another in many characters.

3. More than 125 races were distinguished in culture by the following characteristics: Nature and amount of mycelial growth, color of mycelium, production of pigments in the substratum, zonation, tendency to produce mycelial tufts, "patch-like" growths or variants, and rate of growth.

4. The type of medium has a profound effect on cultural characteristics of *H. gramineum*. Races do not all respond alike. Two races may be similar on one medium but entirely different on another.

5. The association of *H. gramineum* with certain bacteria in culture affects some races differently. It may stimulate or even induce pigment production in certain races, but not in others. Staling products of these bacteria exert differential effects on mycelial development among races.

6. Races of *H. gramineum* also vary in their tolerance to ultraviolet light. This treatment failed to stimulate fructification or to induce production of variants.

7. Some races of *H. gramineum* are stable; others are very unstable. New races arose frequently in culture either from sectors or from "patch" variants. Some of them remained constant through several mycelial transfers, others produced new variants.

8. The results of monosporous reisolation after passing a race back to the host indicate that certain races may give rise to variants while on the living host. Thus the conidial progeny obtained from a barley plant inoculated with a single race fell into 10 distinct cultural groups.

9. The morphology of the conidia may be an additional aid in distinguishing races of *H. gramineum*. There were statistically significant differences in measurements in length, width, and number of septa of conidia between races.

10. At least 20 races, and possibly more, can be distinguished by their relative virulence on 16 varieties of barley. There are profound differences between races in parasitism. Some races are extremely virulent, others moderately so, and still others are only weakly parasitic. A race, however, may attack certain varieties heavily and others weakly, or vice versa.

11. Whenever varieties of barley are inoculated with a combination of races possessing different virulence, the reaction usually corresponds to averages obtained by inoculation of the races singly.

12. In general, Svansota, Manchuria, Minsturdi, Peatland, and Velvet were the most susceptible varieties and were attacked most severely by the largest number of races. No variety, however, was completely susceptible to all of the races. Lion, Glabron, and Wisconsin No. 38 were moderately resistant, while Black Hull-less, Spartan, and Trebi were among the most resistant ones tested.

13. As there are numerous parasitic races of *H. gramineum*, considerable caution is necessary in drawing final conclusions from results on varietal tests.

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